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Remarks

The above Amendments and these Remarks are in reply to the Office Action mailed April 16, 2003.

Claims 32 and 36 have been amended to correct typographical errors, and thus are not narrowing amendments. Claims 38-44 have been amended to correct antecedents and are asserted to be not narrowing amendments.

Claims 11-16, 24-27, 30-31 and 36-46 stand rejected under 35 U.S.C. §102(b) as anticipated and/or obvious under 35 U.S.C. §103 by Sara et al. (EP 0366638; "Sara 1") alone or in combination with the instant specification at pages 1-2 to demonstrate inherency e.g., damage/loss of glial cells resulting from [due to] neural damage/injury e.g., from asphyxia/ischemia/hypoxia/stroke, and dementia disorders such as Alzheimer's addressing non-dopaminergic neurons. Office Action, page 4, paragraph 7. Additionally, "Thus, the reference treatment of neurodegenerative/neurocatabolic disease states and ischemic brain damage (e.g., stroke and asphyxia) addresses the treatment of injuries or disease which result in neural cell death." Office Action, page 7 bridging to page 8. Applicants respectfully traverse the rejections.

I. Anticipation

Applicants submit that Sara 1 does not anticipate, either expressly or inherently, the instant claims. MPEP 2131 states:

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference [references omitted]. The identical invention must be shown in as complete detail as is contained in the ... claim [reference omitted]. Emphasis added.

Express Anticipation

Sara 1 does not disclose "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease comprising the step of administering to said mammal a neuroprotective amount of ... GPE," and therefore doesn't expressly disclose "each and every element as set forth in the claim" as required. The Examiner's statement "[t]hus, the reference treatment of

neurodegenerative/neurocatabolic disease states and ischemic brain damage (e.g., stroke and asphyxia) addresses the treatment of injuries or diseases which result in neural cell death" is unclear. Applicant does not understand whether the term "addresses" was intended to mean "anticipates." Clarification is requested. However, regardless of the meaning of the term "addresses," Applicants assert that altering neurotransmitter release induced by cellular depolarization does not necessarily include treating "injuries or disease which results in neural cell death." Thus, Applicants respectfully submit that Sara 1 does not expressly disclose each and every element of claim 11.

Inherent Anticipation

Applicants submit that Sara 1 does not inherently anticipate the instant claims. MPEP 2112 states:

To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is **necessarily present** in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. **Inherency, however, may not be established by probabilities or possibilities.** The mere fact that a certain thing may result from a given set of circumstances is not sufficient. **Emphasis added.**

Firstly, Applicant's claims are drawn to "methods for treating" and not to "compositions." Applicants note that a claim is drawn to an "invention" and that invention requires a conception and reduction to practice. Applicants note that the discovery of a new effect or new use of a known composition results in a new "conception" and thus a new "invention" that is not necessarily unpatentable. Thus, Applicants' discovery of "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease comprising the step of administering to said mammal a neuroprotective amount of ... CPE" is not necessarily rendered unpatentable by prior art disclosing either GPE or other uses of GPE.

Missing Elements Defeat Anticipation by Inherency

Applicants respectfully submit that there are elements missing from the prior art necessary to link a "neuromodulator" effect of Sara 1 and "neuroprotective" effects of the instant application. Specifically, the words "neuromodulator" and "neuroprotective" do not have the same plain meanings, and are used

differently in the documents themselves. Applicants enclose as Appendix I, copies of relevant pages of the Random House Unabridged Dictionary (Second Edition).

Plain Meanings of "Neuromodulator" and "Neuroprotective"

The term "neuromodulator" is a compound word made of the prefix "neuro," which is understood by persons in the art to refer to neurons. The remainder of the word is "modulator" which is subject to definition by referring to its plain meaning as defined in dictionaries.

The Random House Unabridged Dictionary (Second Edition) defines "modulator" to mean: "A person or thing that **modulates**."

The word "modulate" means:

(1) **to regulate by or adjust to a certain measure or proportion;** soften; tone down. (2) to alter or adapt (the voice) according to the circumstances. (3) *Music* (a) to attune to a certain pitch or key. (b) to vary the volume of (tone). (4) *Telecommunications*: (a) to cause the amplitude, frequency, phase, or intensity of (a carrier wave) to vary in accordance with a sound wave or other signal, the frequency of the signal wave usually being very much lower than that of the carrier. . . . **Emphasis added.**

Likewise, the words "neuroprotective" and "neuroprotection" are compound words consisting of the prefix "neuro" and the remainder being either "protective" or "protection." The Random House Unabridged Dictionary (Second Edition) defines "protection" to mean:

(1) the act of protecting or the state of being protected; **preservation from injury or harm.** (2) **a thing, person, or group that protects:** *This vaccine is a protection against disease. . . .* **Emphasis added.**

Applicants note that the dictionary does not list either as a synonym of the other. Thus, the two terms "modulate" and "protect" have different plain meanings.

Applicants submit that the word "neuromodulate" as used in Sara 1, most closely fits with the definition above "to regulate by or adjust to a certain measure or proportion." The Experiments described in Sara 1 demonstrate that GPE can "regulate or adjust" the function of neurons in brain slices by increasing or inhibiting the release of acetylcholine or by increasing the spinal reflex response.

Applicants also submit that the meaning of the claim limitation “neuroprotective amount of ... GPE” means an amount of GPE that is “neuroprotective.” Applicants further submit that the definition described above applies to the claim language: “preservation from injury or harm” or “a thing . . . that protects.”

The Terms “Neuromodulator” and “Neuroprotective” are Used Differently By Sara and by Applicants

Sara 1 uses the term “neuromodulator” in relation to results of *acute, in vitro* studies on brain slices (e.g., Example 2), in which GPE “is a **modulator** of neural function, thereby **stimulating or inhibiting** neural activity.” Col. 1, lines 36-37; emphasis added. Sara 1 also discloses results of studies showing potentiation of spinal cord reflexes by GPE. Applicants assert that this use of “neuromodulator” is very close to the plain meaning above, namely “to regulate [neurotransmitter release or spinal reflexes] by or adjust to a certain measure or proportion [e.g., by GPE].”

In contrast, Applicants use the term “neuroprotective” refers to inhibition of cell death, as pointed out in claim 11: “A method for **protecting** glial cells or non-dopaminergic neural cells in a mammal **against death** from neural injury or disease comprising the step of administering to said mammal a **neuroprotective** amount of . . . GPE...” Emphasis added. Applicants submit that their use of “neuroprotection” is close to the above definition: “the act of protecting or the state of being protected; **preservation from injury or harm. (2) a thing, person, or group that protects: *This vaccine is a protection against disease. . . .***” [Emphasis added, italics in original.]

For Sara 1 to inherently anticipate the instant claims, persons of ordinary skill would have to believe that **both increasing and decreasing** neurotransmitter release are **necessarily** linked to “protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease.” If the Examiner is aware of any evidence of such a reasonable belief, he is requested to provide such evidence, through either a prior art reference if available, or an Affidavit or a Declaration.

Further, Applicants invite consideration of what is not disclosed in Sara 1. Although Sara 1 discusses **potential** uses of GPE to treat dementias, Sara 1 does not provide an enabling disclosure of any such uses. Sara 1 discloses no experiments on neural survival. Sara 1 discloses (1) no *in vivo* experiments

in which GPE was used, (2) no long-term studies of any effect of GPE, (3) no experiments in which neural survival *in vivo* or *in vitro* was measured, and (4) no link between acute *in vitro* studies on acetylcholine release or spinal cord reflexes and survival of any cell type. Further, (5) none of the studies were described as being on brain slices from any animal that had been subjected to neural damage or disease. Thus, Applicants conclude that Sara 1 did not describe any conception and reduction to practice of any "neuroprotective" effect of GPE, and therefore cannot anticipate the instant claims.

Finally, the Examiner has provided no evidence that necessarily links "stimulating or inhibiting neural activity" with "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease" as in claim 11. [Emphasis added.]

Therefore, Applicants submit that Sara 1 cannot inherently indicate that neuromodulation is useful for "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease" Although it may be possible that such an effect exists, such a **possibility** cannot sustain a rejection based on inherency. "Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient." MPEP, *Id.* Thus, Applicants submit that a *prima facie* case for anticipation has not been made.

In light of the dearth of enabling disclosure about roles of GPE on neuroprotection, Applicants submit that Sara 1 cannot anticipate the instant claims.

II. Obviousness

A. Sara

Claims 11-17, 24-27, 30-31 and 36-46 stand rejected under 35 U.S.C. §103 as obvious over Sara (Sara 1).

To establish a *prima facie* case for obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a **reasonable expectation of success**. Finally, the prior art reference must **teach or suggest all the claim limitations**. The teaching or suggestion to make the claimed combination

and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure.

Applicants respectfully submit that the instant claims cannot be rendered obvious by Sara 1. As described above, Sara 1 discloses that GPE either "stimulates or inhibits" neural activity and can potentiate spinal cord reflexes. However, Sara 1 neither teaches nor suggests that GPE can be effective in "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease." [Emphasis added.] Applicants therefore submit that Sara 1 cannot render the instant claims obvious.

Thus, at best, the experiments disclosed in Sara 1 provide an "invitation to experiment" on possible effects of GPE on brain slices from brain-damaged or brain-diseased animals, but could not have provided a reasonable basis to conclude that acute effects of GPE on neurotransmitter release inherently discloses any property of GPE to promote "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease." Rather, for Sara 1 to render the instant claims obvious, both potentiation and inhibition of acetylcholine release would have to relate to "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease". No link between neurotransmitter release and either disease or cell death was made in Sara 1, nor was there any disclosure of conception or understanding that intervention using GPE could result in decreased neural cell death (neuroprotection).

Regarding the fact that a *prima facie* case for obviousness requires a "motive to modify the reference" and "reasonable likelihood of success," Applicants note that a subsequently published article by Sara ("The Biological Role of Truncated Insulin-like Growth Factor-1 and the Tripeptide GPE in the Central Nervous System" *Annals of the New York Academy of Science*; pp: 183 - 191 (1991); "Sara 2"; copy enclosed in Appendix II) addresses similar issues as in the Sara EP 0366638 ("Sara 1") but actually teaches away from Applicants' claims. In particular, Sara 2 states:

Extensive *in vivo* studies have **not revealed any growth-promoting activity of GPE**. . . . As shown in Figure 4, no significant growth effects, including tail length and organ weights, were observed. Page 187, middle of first full paragraph.

Thus, Applicants submit that at the time of publication of Sara 2, the first inventor of Sara 1 (Sara) could not have had a reasonable belief that GPE could be a growth modulator, and thus that there would be neither a motive to try nor a reasonable likelihood of success at achieving the Applicants' invention. In the absence of a reasonable belief by the primary inventor, Applicants submit that no person of ordinary skill could have such a reasonable belief. Applicants submit that both Sara 1 and Sara 2 considered GPE to be an agent that acted on neurotransmitter receptors and not as a growth promoting hormone. Because Sara 1 was silent about any effects of GPE to promote "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease," Applicants submit that at the time of publication of Sara 1, there was neither motive nor a reasonable belief that GPE could so act.

Rather, Applicants respectfully submit that the motive and reasonable likelihood of success were provided by the Applicant's own instant disclosure.

B. Sara in View of Sibalis

Claims 11-17, 24-27, 30-31 and 36-46 stand rejected under 35 U.S.C. §103 over Sara (Sara 1) in view of Sibalis (U.S. 5,032,109; "Sibalis").

Applicants incorporate herein the discussions presented above for Sara 1.

The Examiner stated that Sibalis teaches transdermal delivery of "polypeptides containing about three to 20 alphaamino acid units." However, Applicants can find no teaching in Sibalis and Sara 1 together of any method for "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease." Thus, the combination of Sara 1 and Sibalis does not disclose all the limitations of the pending claims with a reasonable likelihood of success, and thus cannot render Applicants' claims obvious. Applicants therefore urge the Examiner to reconsider the rejection and find the claims allowable.

C. Sara in View of Gluckman

Claims 11-16 and 18-46 stand rejected under 35 U.S.C. §103 over Sara (Sara 1) in view of Gluckman (WO 93/02695; "Gluckman").

Applicants incorporate herein the discussions presented above for Sara 1.

The Examiner stated that Gluckman teaches "a method for treatment or prevention of CNS damage caused by neurodegenerative disease and trauma which primarily causes damage to glia and/or other non-cholinergic cells in the CNS." Office Action, page 9, bottom paragraph. The Examiner also stated "It is noteworthy that the Gly-Pro-Glu peptide, as presently claimed, is derived from the N-terminal three amino acids of IGF-1 peptide." Office Action, page 10 bottom of first paragraph.

Applicants note that Gluckman does not disclose "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease, comprising administering a neuroprotective amount of ... GPE ..." as in claim 11. Nowhere in either Sara 1 nor Gluckman, nor in the combination of Sara 1 and Gluckman together, is any teaching of the use of GPE as in claim 11. Thus, the combination of Sara 1 and Gluckman does not disclose all the limitations of the pending claims with a reasonable likelihood of success, and thus cannot render Applicants' claims obvious. Although GPE is the N-terminal tripeptide of IGF-1, both Sara 2 and Gluckman teach away from GPE as a neuroprotective agent. First, Gluckman teaches that IGF-1 is neuroprotective (e.g., see Abstract and Summary of the Invention, page 3, first paragraph). Next, Sara 2 states: "The aminoterminal tripeptide of IGF-1, GPE, displays a different range of biological actions compared to truncated IGF-1. These effects are not mediated by IGF-1 receptors. As shown in Figure 3, GPE fails to cross-react with the IGF-1 receptor and does not influence the binding of either intact or truncated IGF1 to the receptor." Page 187, middle paragraph, middle section. Thus, Applicants submit that one of ordinary skill in the art would view Sara 1 in the same light as Sara 2, and when combined with Gluckman, would provide no motive to nor a reasonable believe in the success of, any study to determine whether GPE had neuroprotective properties.

Rather, Applicants submit that the instant disclosure provided the link between IGF-1, GPE and "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease, comprising administering a neuroprotective amount of ... GPE ..." as in claim 11. "To date, there has been no enabling reference in the prior art to the manipulation of the cleaved tripeptide GPE itself to prevent or treat CNS injury or disease leading to CNS damage *in vivo*." Page 3, third paragraph. Using such hindsight reconstruction to argue for unpatentability is impermissible under 35 U.S.C. §103, the MPEP

and case law. Applicants therefore urge the Examiner to reconsider the rejections and find the claims allowable.

III. Conclusions

Applicants respectfully submit that there is insufficient showing that Sara 1 either expressly or inherently anticipates or renders the instant claims obvious and that no *prima facie* for either rejection has been made. Applicants respectfully request the Examiner to provide the missing evidence necessary to make a *prima facie* showing of either anticipation or obviousness. In the absence of such evidence, either through citation of a publication or through an Affidavit or Declaration, Applicants request the Examiner to reconsider the rejections and find the claims allowable.

Further, Applicants conclude that no combination of Sara 1, Sibalis or Gluckman taught or suggested, with a reasonable likelihood of success, all limitations of the instant claims, and therefore, that no combination of those references renders the instant claim obvious. In fact, Sara 2 actually taught away from the instant claims. Because Sara 2 was published after Sara 1, Applicants conclude that any interpretation of Sara 1 to teach "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease, comprising administering a neuroprotective amount of ... GPE ..." is not supported.

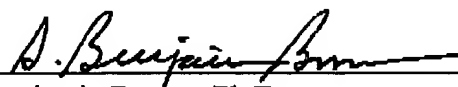
In light of the above, it is respectfully submitted that all of the claims now pending in the subject patent application should be allowable, and a Notice of Allowance is requested. The Examiner is respectfully requested to telephone the undersigned if he [she] can assist in any way in expediting issuance of a patent.

The Commissioner is authorized to charge any underpayment or credit any overpayment to Deposit

Account No. 06-1325 for any matter in connection with this response, including any fee for extension of time, which may be required.

Respectfully submitted,

Date: July 10, 2003

By: 
D. Benjamin Borson, Ph.D.
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APPENDIX I

**Copies of Relevant Pages from
Random House Unabridged Dictionary (Second Edition)**

RANDOM HOUSE UNABRIDGED DICTIONARY

Second Edition

atic variation.

o-der-sohn-Beck-er (mō'dən zōn bek'ər), n. *Pa-*
i (pou'la), 1878-1907, German painter**od-est** (mōd'ist), adj. 1. having or showing a mod-
erate or humble estimate of one's merits, importance,
etc.; free from vanity, egotism, boastfulness, or great
retensions. 2. free from ostentation or showy extra-
vance: a modest house. 3. having or showing regard for
the decencies of behavior, speech, dress, etc.; decent: a
modest neckline on a dress. 4. limited or moderate in
amount, extent, etc.: a modest increase in salary. [1555-
56; < L *modestus* restrained, decorous, equiv. to *moder-*
at of "modus, an s-stem akin to *modus* MODE, perh. <
modos, with the vowel of *modus*; cf. *moderari* to *mod-*
erate, from the same n: stem) + -us adj. suffix]
-mod'est-ly, adv.—Syn. 1. retiring, unassuming. 1, 2. unpretentious,
unobtrusive. 3. pure, virtuous. *Modestus*, *decurus*, *rat-*
on imply conformity to propriety and decorum, and a
latitude for anything coarse or loud. *Modest* implies a
recoiling shyness, sobriety, and proper behavior: a mod-
est self-respecting person. *Deurus* implies a bashful,
quiet simplicity, staidness, and decorum; but can also in-
dicate an assumed or affected modesty: a demure young
lady. *Pravus* suggests an exaggeratedly self-con-
scious modesty or propriety in behavior or conversation
of one who wishes to be thought of as easily shocked and
who often is intolerant: a prudish objection to a harmless
remark. —Ant. 3. bold, coarse.**lo-das-to** (mō dās'tō), n. a city in central California.
06,105.**mod-est-ty** (mōd'ə stē), n. pl. -ties. 1. the quality of
being modest; freedom from vanity, boastfulness, etc. 2.
regard for decency of behavior, speech, dress, etc. 3.
implicit, moderation. [1525-35; < L *modestia*. See
COPEST, -r]**mod'esty pan'el**, a panel across the front of a desk,
esp. an office desk, designed to conceal the legs of a per-
son seated at it.**MODFET** (mōd'fēt), n. Electronics. modulation-doped
field effect transistor.**modGk**, Modern Greek. Also, *Mod. Gk.*, *Mod. Gr.***modHeb**, Modern Hebrew. Also, *Mod. Heb.***mod-i-cum** (mōd'i kəm), n. a moderate or small
amount. He hasn't even a modicum of common sense.
1425-75; late ME < L n. use of neut. of *modicus* moder-
ate, equiv. to *modi-*, comb. form of *modus* limit (see
MODE) + -us adj. suffix]**modif.**, modification.**mod-i-fi-cand** (mōd'ə fī kənd'), n. *Gram.* a word that
is modified, or qualified, by another. In red books, books
a modificant. [1825-35; < L *modificantum* (a thing)
to be measured or limited, ger. of *modificare* to modify]**mod-i-fi-ca-tion** (mōd'ə fī kə'shən), n. 1. an act or
instance of modifying. 2. the state of being modified;
partial alteration. 3. a modified form; variety. 4. Biol.
a change in a living organism acquired from its own ac-
tivity or environment and not transmitted to its descend-
ants. 5. limitation or qualification. 6. *Gram.* a. the use
of a modifier in a construction, or of modifiers in a class
of constructions or in a language. b. the meaning of a
modifier, esp. as it affects the meaning of the word or
other form modified: Limitation is one kind of modifica-
tion. c. a change in the phonological shape of a mor-
pheme, word, or other form when it functions as an ele-
ment in a construction, as the change of *not* to *n't* in
doesn't. d. an adjustment in the form of a word as it
passes from one language to another. [1495-1505; < L
modificatio (s. of *modificatio*), equiv. to *modificatus* (us)
(pp. of *modificare*; see *MODIFY*) + -iōn]**mod-i-fi-ca-tory** (mōd'ə fī kə'tōr'ē), adj.
modifying. Also, *mod-i-fi-ca-tive*. [1815-25; < L
modificatus (us) (see *MODIFICATION*) + -ory]**mod-ified Amer-ican plan**, (in hotels) a system of
paying a single fixed rate that covers room, breakfast,
and one other meal, usually dinner. Abbr.: MAP Cf.
American plan, demi-pension, European plan.**mod-i-fi-er** (mōd'ə fī'ər), n. 1. a person or thing that
modifies. 2. *Gram.* a word, phrase, or sentence ele-
ment that limits or qualifies the sense of another word,
phrase, or element in the same construction. b. the im-
mediate constituent of an endocentric construction that
is not the head. [1575-85; *MODIFY* + -er]—Usage. See dangling participle, misplaced
modifier.**mod-i-ty** (mōd'ə tē), v., -fied, -fying. —u.t. 1. to
change somewhat the form or qualities of; alter partially;
amend: to modify a contract. 2. *Gram.* (of a word,
phrase, or clause) to stand in a syntactically subordinat-
ing relation to (another word, phrase, or clause), usually
with descriptive, limiting, or particularizing meaning; be
a modifier. In a good man, good modifies man. 3. to be
the modifier or attribute of. 4. to change (a vowel) by
umlaut. 5. to reduce or lessen in degree or extent; mod-
erate; soften: to modify one's demands. —u.t. 6. to be
become modified. [1350-1400; ME *modifien* < MF
modifier < L *modificare* to impose a rule or pattern,
regulate, restrain. See *MODER*, -ry] —mod-i-ty-ble, adj.
adj. —mod-i-ty-ble-ly, mod-i-ty-ble-ness, n.—Syn. 1. vary, adjust, shape, reform. 3. *MODIFY*,
QUALIFY, TEMPER suggest altering an original statement,
condition, or the like, so as to avoid anything excessive
or extreme. To *MODIFY* is to alter in one or more particu-
lars, generally in the direction of leniency or moderation;
to modify demands, rates. To *QUALIFY* is to restrict or
limit by exceptions or conditions; to qualify one's praise.

mod-i-ty

mod-i-o-lus (mō dī'ō ləs, mō-), n. pl. -li (-lī'). Anat.
the central, conical axis of the cochlea of the ear. [1685-
95; < NL *L.* nave of a wheel bucket, drinking vessel,
equiv. to *modi* (us) a dry measure (perh. deriv. of *modus*
MODE) + -ulus (-ulus) —mod-i-o-lar, adj.**mod-ish** (mō'dish), adj. in the current fashion; stylish.
[1850-60; *MODE* + -ish] —mod-ish-ly, adv. —mod-
ish-ness, n.

—Syn. smart, chic, fashionable, trendy.

mod-iste (mō dēst; Fr. mō dēst'), n. pl. -istes
(-dēst'; Fr. -dēst'). Older Use. a female maker of or
dealer in women's fashionable attire. [1830-40; < F; see
MODE, -ist]**Mod-jes-ka** (mō jēs'kə), n. *Mo-jes-ka* (ho jēs'kə), (Hel-
ena Opłód Modzejewska), 1840-1909, Polish actress, in
U.S. after 1876.**Mod-oc** (mō'dok), n. pl. -docs, (esp. collectively) -doc.
a member of an American Indian people belonging to the
Lutunian group and ranging from southern Oregon to
northern California.**mod-ock wool** (mō'dok). See territory wool. [spe-
cial use of *MOOC*]**mod. praesc.**, (in prescriptions) in the manner pre-
scribed; as directed. [< L *modo praescripto*]**Mod-red** (mō'drid), n. Arthurian Romance. the
nephew and treacherous killer of Arthur. Also, *Mod-*
red.**mod-u-lar** (mōj'ə lər), adj. 1. of or pertaining to a
module or a modulus. 2. composed of standardized units
or sections for easy construction or flexible arrangement:
a modular home; a modular sofa. 3. Math. (of a lattice)
having the property that for any two elements with one
less than the other, the union of the smaller element
with the intersection of the larger element and any third
element of the lattice is equal to the intersection of the
larger element with the union of the smaller element and
the third element. 4. Computers. composed of software
or hardware modules that can be altered or replaced
without affecting the remainder of the system. —n. 3.
something, as a house or piece of furniture, built or or-
ganized in self-contained units or sections. 6. a self-con-
tained unit or item, as of furniture, that can be combined
or interchanged with others like it to create different
shapes or designs. [1790-1800; < NL *modularis*. See
MODULE, -AR]**mod-ular arith-metic**, arithmetic in which numbers
that are congruent modulo a given number are treated
as the same. Cf. congruence (def. 2), modulo, modulus
(def. 2b). [1955-60]**mod-ular-ity** (mōj'ə lar'itē, mōd'yə-), n. the use of
individually distinct functional units, as in assembling an
electronic or mechanical system. [1935-40; *MODULAR* +
-ity]**mod-u-lar-ize** (mōj'ə lə'riz'), v., -ized, -izing. to
form or organize into modules, as for flexibility. Also,
esp. Brit., *mod-u-lar-ize*. [1955-60; *MODULAR* + -ize]
—mod-u-lar-ize-ation, n.**mod-u-lar-ize** (mōj'ə lə'riz'), v., -ized, -izing. —u.t. 1.
to regulate by or adjust to a certain measure or propor-
tion; soften; tone down. 2. to alter or adapt (the voice)
according to the circumstances, one's listener, etc. 3.
Music a. to attune to a certain pitch or key. b. to vary
the volume of (tone). 4. Telecommunications. to cause
the amplitude, frequency, phase, or intensity of (a car-
rier wave) to vary in accordance with a sound wave or
other signal, the frequency of the signal wave usually
being very much lower than that of the carrier. —u.t. 5.
Telecommunications. a. to modulate a carrier wave. b.
CB Slang. to talk; visit. Enjoyed modulating with you.
6. Music. to pass from one key to another: to modulate
obruptly from A to B flat. [1550-60; < L *modularis*
(s. of *modulari*) to regulate (sounds), set to music, play
an instrument. See *MODULE*, -ate] —mod-u-lar-ize-ation, n.
(mōj'ə lə'riz'ē), n. —mod-u-lar-ize-ative, mod-u-lar-ize-
-ory (mōj'ə lə'riz'ē, -tōr'ē), adj.—Syn. 2. temper, control.
mod-u-la-tion (mōj'ə lə'shən, mōd'yə-), n. 1. the act
of modulating. 2. the state of being modulated. 3.
Music. transition from one key to another. 4. *Gram.* a.
the use of a particular distribution of stress or pitch in
a construction, as the use of rising pitch on *here* in *John is*
here? b. the feature of a construction resulting from
such use. [1360-1400; ME < L *modulatio* (s. of *modu-*
latus) rhythmic measure. See *MODULATE*, -ION]**mod-u-la-tor** (mōj'ə lə'tər), n. 1. a person or thing
that modulates. 2. Telecommunications. a device for
modulating a carrier wave. [1490-1500; < L *modulator*;
see *MODULATE*, -OR]**mod-ule** (mōj'ool), n. 1. a separable component, fre-any two operators and any group element in
having the first operator act on the elemen-
second element, and the second operator act
on element is equal to the result of having a
erator, formed by adding or multiplying the
tors, act on the first element. Cf. ring' (de
Computers. a. part of a program that perform
function. b. an interchangeable, plug-in hard
[1555-65; < L *modulus*; see *MODULUS*]**mod-u-lus** (mōj'ə lō'), adv. Math. with re-
modulus 6 is congruent to 11, modulo 6. [181
NL *modulus*, abl. of L *modulus* MODULUS]**mod-u-lus** (mōj'ə lō'), n. pl. -li (lī). 1. Phy-
efficient pertaining to a physical property. 2
that number by which the logarithms in one
multiplied to yield the logarithms in another.
tity by which two given quantities can be
yield the same remainder. c. See *Abso-*
[1555-65; < L a unit of measure; see *MOOD*]**mod-ulus of elastic-ity**, Physics. any of
efficients of elasticity of a body, expressing the
tress a stress or force per unit area that acts
the body and the corresponding fractional d
caused by the stress. Also called coefficient
ity, elastic modulus. [1800-10]**mod-ulus of rigid-ity**, Physics. See *shear*
[1875-80]**mod-ulus of tor-sion**, Physics. See *shear*
[1875-80]**mod-ulus of op-er-ant** (mō'dəs op'ə ran'dē
mō'dəs op'ə ran'dē), pl. *mod-i op-er-ant*
op'a ran'dē, mō'di op'a ran'dē; Lat. mō'dē
dē). mode of operating or working. [1641
modus operandi]**mod-ulus vi-ven-dē** (mō'dəs vi ven'dē, -dē),
vi-ven-dē (mō'dē vi ven'dē, mō'dē vi ven'dē),
ner of living; way of life; lifestyle. 2. a term
rangement between persons or parties pend-
ment of matters in debate. [1875-80 < L *modus*
mode of living]**Mod** (mō), n. a male given name, form of
Moses.**Mod-ius** (mō'bē s, mō'-, mō'-), n. Aug-
and. See *Modius*, August Ferdinand.**Mod-ras** (mō'rās), n. pl. *Class. Myth.* the Fat
Moe-si-a (mō'shē-ā), n. an ancient country;
rope, S of the Danube and N of ancient Thrace;
odonia; later a Roman province.**Mod-so-goth** (mō'sō goth'), n. one of
tized Goths who settled in Moesia in the 4
A.D.**Mod-so-goth-ic** (mō'sō goth'ik, -sē-), adj.
taining to the Moesogoths or their language
goth + -ic]**mo-fette** (mō fēt'; Fr. mō fēt'), n. 1. a noi-
nation, consisting chiefly of carbon dioxide
from the earth in regions of nearly extinct v-
tivity. 2. one of the openings or fissures from
emanation issues. Also, *mo-fette*. [1815-25
mo-fette (Neapolitan *mo-fette*), equiv. to *muff*
It *muffa* mould (< Langobardic; cf. G *Muff*;
MHG *Muffeln* to give off a foul smell) + -ette**mog** (mog), v., mogged, mog-ging. Dial. -
move on, depart, or decamp (usually fl. by
2. to walk or move along gently, slowly, an-
t. 3. to cause to go from one place to
[1885-75; *mog* (< *mog*)]**mog** (mog), n. moggy. [by shortening]**Mog-a-di-shu** (mō'gā dē'shō), n. a seap-
the capital of Somalia, in the S part. 400,000. In
ga-di-shu (mō'gā dē'shō).**Mog-a-dor** (mō'gā dōr', -dōr'; Fr. mō gā dō
former name of *Essaouira*. 2. (L.) Also, mo-
a ribbed fabric of silk or rayon warp and cotton
filling, used for neckties.**Mog-en Da-vid** (mō'gən dā'vid; Seph. *Hel*
dā vōd'; Ashk. Heb. mō'gən dā'vid), Jude
Star of David. [1900-05]**mog-gy** (mog'gē), n. pl. -gies. Brit. Inform.
Also, *mog*. [1815-25; said to be orig. Cockney
derivations from dial. (W Midlands) *Moggy* pe
a calf, or from personal name *MAGGIE*, are di
Mog-hul (mō'gūl, -gūl, mō gūl'), n., adj. M
1, 2, 6.]**Mog-l das Cru-zes** (mō'zō dās kruz'ē), n.
in SE Brazil, E of São Paulo. 111,654.**mog-i-la-ka** (mōj'ə lā'ka, -lā'yē), n. any
feet, as stuttering or stammering. Also, *mo-lia*
[1850-60; < Gk *mogilē* (as) hardly talking (*mogilē*)
culty + *lālos* babbling) + -ia -ia]**Mog-i-lev** (mōj'ē lēf'; Russ. mō gyl'ēf'), n.
E Belyorussia (Belarus), on the Dnieper. 359,
E of Belyorussia (Belarus), on the Dnieper. 359,**mo-go** (mō'gō), n. pl. -gos. Australian.
hatchet used by the Aborigines. [1815-25;
mu-gu]**Mog-gu-lon** (mō'gō yōn'), n. 1. an exten-
sive mesa in central Arizona; the southwestern
Colorado Plateau. 2. a mountain range
Mexico. —adj. 3. Archaeol. of or pertaining to
Indian culture of southeastern Arizona and
New Mexico 100 A.D.-A.D. 1000, character-
houses also used for burials and a distinctive
white pottery decorated with human and animal

ric, adj.	pro-Phil/ p-pine/, adj.
-ism, n.	pro-Pol/ish, adj.
age, adj.	pro-pol/i-tics, adj.
it, adj.	pro-/Panta-guess/, adj., n.
u,	

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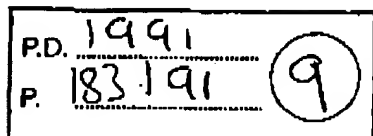
expression of protactinium.

APPENDIX II

Copy of Reference

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**The Biological Role of Truncated Insulin-like Growth Factor-1
and the Tripeptide GPE in the Central Nervous System**



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The Biological Role of Truncated Insulin-like Growth Factor-1 and the Tripeptide GPE in the Central Nervous System^a

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INTRODUCTION

Since early in this century, attempts have been made to identify substances present in serum and organ extracts that are capable of promoting the growth of the nervous system. Today several such growth-promoting factors have been isolated and identified, such as nerve growth factor (NGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and insulin-like growth factors (IGFs). It has become clear that these growth factors are endogenously produced within the developing nervous system at specific growth phases and that they interact to regulate the growth and development of the central nervous system (CNS). A role for the IGFs in the regulation of CNS growth was first implicated 20 years ago by the finding that growth hormone had an indirect action on brain growth which was believed to be mediated by the production of a brain growth factor from either the placenta or the fetus.^{1,2}

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The presence of IGF-1 in the nervous system appears to be a phylogenetically ancient phenomenon. Using immunological methods, IGF-1 has been localized in the nervous system as well as the gut of lower vertebrates, including bony and cartilaginous fish and cyclostomi, as well as protochordates. For example, IGF-1 immunoreactive perikarya and fibers have been observed in all levels of the brain of the Atlantic hagfish, *Myxine glutinosa*.⁸ IGF-1-like immunoreactivity has also been localized in central neurones of the urochordate *Ciona intestinalis* and the cephalochordate *Branchiostoma lanceolatum*.⁹ Thus the presence of IGF-1 in the "brain-gut axis" has been well preserved during vertebrate evolution. The identity of the IGF-1-like molecule in the brain-gut axis of the lower vertebrates and protochordates remains to be determined. The nucleotide sequence of an IGF cDNA isolated from *Myxine glutinosa* showed 70% homology to the A and B domains of both human IGF-1 and IGF-2,¹⁰ and a hybrid insulin/IGF cDNA related to both human insulin and IGFs has been cloned from *Branchiostoma californiensis*.¹¹ Chan *et al.* have proposed that the latter hybrid molecule represents the transiogen form prior to insulin and IGF divergence at an early stage of vertebrate evolution.¹¹ It is of interest that the deduced amino acid sequence of the hybrid insulin/IGF molecule reveals a different aminoterminal dipeptide compared to mammalian IGF-1. A plasma membrane receptor similar to that of mammalian IGF-1 receptor has also been identified in the nervous system of lower vertebrates, including *Myxine glutinosa*.¹²

The truncated IGF-1 has been identified in several tissues (FIGURE 1). Ogasawara *et al.* identified truncated IGF-1 in porcine uterus where the peptide accounted for the complete mitogenic activity of uterine extracts.¹³ Truncated IGF-1 has also been isolated from human platelets.¹⁴ Lysates of human platelets contain intact as well as truncated IGF-1 and IGF-2. IGF-1 was released from the platelets during degranulation, suggesting a role in wound healing.¹⁵ Francis *et al.* have identified truncated IGF-1 in bovine colostrum where intact IGF-1 was additionally found to be present.¹⁶ In all studies during its purification, truncated IGF-1 displayed enhanced biological activity. With the availability of synthetic and recombinant truncated IGF-1, the reason for this enhanced biological potency became apparent. Truncated IGF-1 binds only weakly to the IGF binding proteins (IGFBPs).¹⁷⁻²⁰ Although truncated IGF-1 shows some binding to IGFBP-3, a marked reduction in binding affinity to IGFBP-1, -2, -6 has been found in comparison to intact IGF-1 (less than 1%). Analogues of IGF-1 with substitutions in the aminoterminal pentapeptide have identified Glu in residue 3 as playing a significant role in IGFBP binding. In a wide variety of cultured cells, it has been demonstrated that the enhanced biological activity of the truncated IGF-1 is most likely due to its failure to be bound by IGFBPs which can compete with the IGF-1 receptor and attenuate the biological activity of IGF-1. Thus the failure to bind to IGFBPs results in a greater availability of truncated IGF-1 to the target cell receptors (FIGURE 2).

BIOLOGICAL ACTION IN CNS

Truncated IGF-1 (-3N:IGF-1) and the tripeptide GPE display separate biological functions in the nervous system which are mediated via distinct receptors on their target cells. Truncated IGF-1 has a potent neurotrophic action via interaction in the IGF-1 receptor in the CNS. The IGF-1 receptor is widely distributed throughout the CNS,²¹ and its expression is enhanced during the rapid growth phase of early life. IGF-1's neurotrophic action predominates during early development, when the IGFs regulate the growth and differentiation of the nervous system. *In vitro* studies have demonstrated that IGF-1 stimulates the proliferation of neuroblast and glioblast

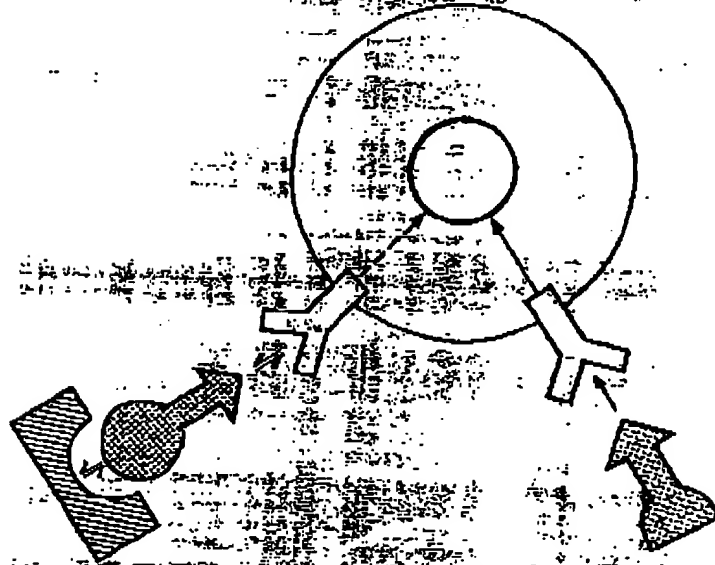


FIGURE 2. Model to account for the reduced neurotrophic activity of truncated IGF-1 compared to intact IGF-1. Failure to become complexed with the IGFBPs is hypothesized to result in greater availability of truncated IGF-1 to the IGF-1 receptors on target cells.

precursor cells, as well as their differentiation.²³ Several studies indicate that IGF-1 may play an important role in synapse formation and myelination.²⁴⁻²⁷ Comparison between the various IGFs reveals that truncated IGF-1 displays a far greater neurotrophic activity compared to intact IGF-1 or IGF-2. Using fetal brain cells *in vitro*, Carlsson-Skewir *et al.* showed that this enhanced neurotrophic potency was due to failure of truncated IGF-1 to bind to binding proteins.²⁸ The addition of IGFBP-1 to the culture medium could inhibit the stimulation of fetal brain cell DNA synthesis by intact IGF-1 but had no effect on the action of truncated IGF-1. A similar action has been observed with intracellular transplantation of brain tissue by Giacobini *et al.*²⁹ Truncated IGF-1 had a potent growth-promoting action on parietal cortex and spinal cord grafts transplanted into the anterior chamber of the eye of adult rats. This *in vivo* model allows for direct observation of graft survival and growth. The action of truncated IGF-1 was developmentally as well as regionally specific. For example, a significant growth-promoting action was observed in spinal cord grafts at embryonic day E14 but not at E16. It was suggested that these differences reflected the stage of receptor maturation and function in the various regions. This is in accordance with the expression of the IGF-1 gene in specific cell populations at discrete times during CNS development.²³ Intact IGF-1 failed to stimulate the growth of brain transplants, presumably due to the presence of IGFBPs which were present in the vitreous fluid.

Similarly, when administered intravenously, truncated IGF-1 displays reduced binding to IGFBPs in the circulation. The truncated form is more rapidly cleared

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from the blood²² and is also degraded faster than intact IGF-1.³¹ Consequently, the acute hypoglycemic effect of truncated IGF-1 is greater than that of intact IGF-1.³² Increased degradation due to low association with IGF-BPs most likely explains the failure to observe any significant enhancement in growth following the subcutaneous administration of truncated IGF-1 to neonatal rats in spite of enhancement being observed following intact IGF-1 administration. In contrast to the growth of normal rats, enhanced growth has been observed in growth-hormone-deficient *h/h* mice following truncated IGF-1 administration.³³ Truncated IGF-1 has similarly been reported to be more potent than intact IGF-1 in regulating nitrogen balance and muscle protein metabolism in nitrogen-restricted rats.³⁴

The aminoterminal tripeptide of IGF-1, GPE, displays a different range of biological actions compared to truncated IGF-1.³⁵ These effects are not mediated by IGF receptors. As shown in FIGURE 3, GPE fails to cross-react in the IGF-1 receptor and does not influence the binding of either intact or truncated IGF-1 to the receptor. The tripeptide similarly fails to cross-react in the IGF-2 receptor. GPE does not bind to IGF-BPs nor does it influence the association of the IGFs to their binding proteins. Extensive *in vivo* studies have not revealed any growth-promoting activity of GPE. The results of one such study are summarized in FIGURE 4. Growth was followed in rats receiving 30 µg GPE subcutaneously (sc) per day from days 3 to 15 of postnatal life. As shown in FIGURE 4, no significant growth effects, including tail length and organ weights, were observed. However at maturity, the animals receiving GPE during this preweaning period displayed a significant increase in activity measurements in an open field test. It has since been demonstrated that GPE plays a neuromodulatory role in the CNS,³⁶ which may account for the changes in activity observed in the GPE-treated rats.

The structure of GPE suggested that it may interact in receptors for glutamate which is a major excitatory amino acid neurotransmitter in the CNS. Using rat synaptic membranes, it was shown that GPE cross-reacted in the *N*-methyl-D-

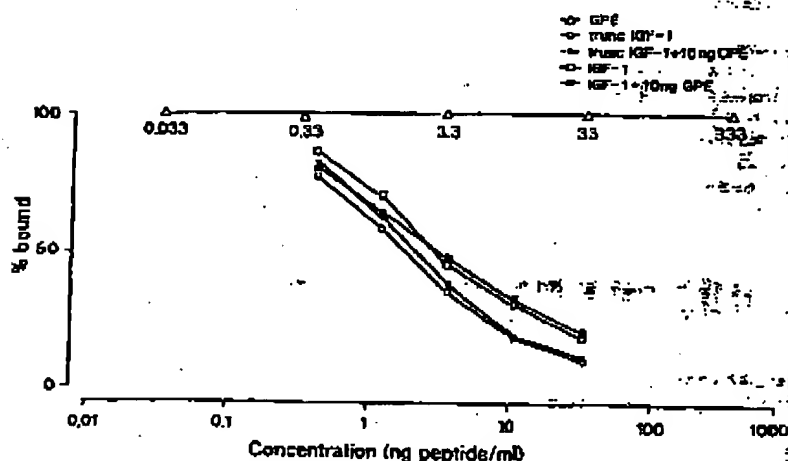


FIGURE 3. Competition with ¹²⁵I-IGF-1 for binding to human fetal brain membranes. Data are expressed as the percentage bound in the absence of competing peptide.

aspartate (NMDA) but not the kainate or quisqualate type of glutamate receptor.³⁵ While the carboxyl terminal glutamate was necessary for NMDA receptor binding, the aminoterminal glycine potentiated this excitatory reaction. Glycine has earlier been shown to potentiate responses mediated via the NMDA receptor and has been suggested to be a specific regulator of the NMDA receptor via binding to an allosteric site.³⁶ GPE facilitates the release of dopamine from cortical slices. As shown by the use of a selective competitive antagonist, this action is mediated via interaction in the NMDA receptor.³⁷ In addition, GPE has a potent stimulatory action on acetylcholine release from cortical neurones.^{31,37} This action cannot be inhibited by NMDA receptor blocking agents, and acetylcholine potentiation is mediated via an as yet unidentified receptor. GPE does not interact with choline uptake sites or muscarinic receptors on neurones. Although GPE interacts in nicotinic binding sites in the rat cortex, this interaction occurs at a concentration several orders of magnitude greater than that required to potentiate acetylcholine release. Thus the receptor mechanism for acetylcholine potentiation remains to be identified. It had been reported earlier that intact IGF-1 potentiates acetylcholine release from cortical slices.³⁸ Similarly, IGF-1 has been reported to enhance catecholamine release from chromaffin cells.³⁹ However, truncated IGF-1 fails to enhance acetylcholine release from the cortical slice.³⁹ It may be suggested that the action of intact IGF-1 on neurotransmitter release was due to the tripeptide GPE. However, during this acute experiment, significantly less truncated IGF-1 was taken up by the cortical slices during the 30-minute incubation period. Thus, in the adult cortex, formation of the IGFBP complex may be necessary for transport across the microvascular barrier to directly interact with neurones and induce an acute action. Neuronal activity can be modulated by GPE. This has been demonstrated in single cortical neurones following iontophoretic application of GPE. As shown in FIGURE 5, GPE alone has no effect on the electrophysiological activity of the neurone; however, when applied together with glutamate, it potentiates the action of

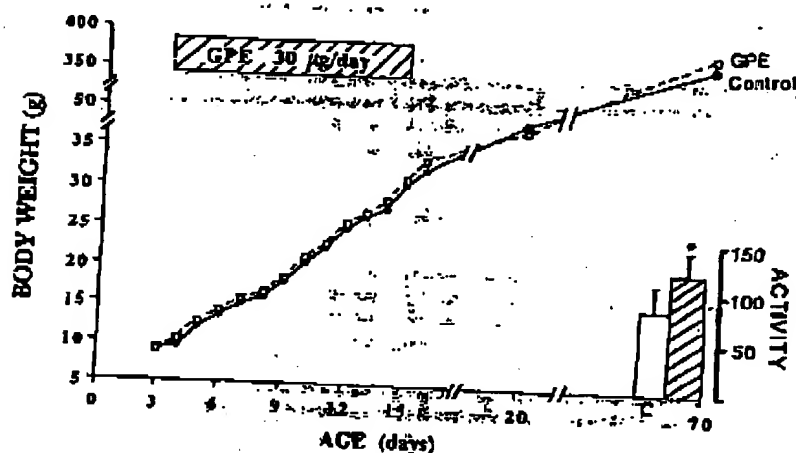


FIGURE 4. The growth of rats receiving either 30 µg GPE sc/day or vehicle alone from days 3 to 15 of postnatal life. No significant effect on body weight was observed. At 70 days of age, open field behavior was examined. GPE-treated rats displayed a significant increase in activity scores.

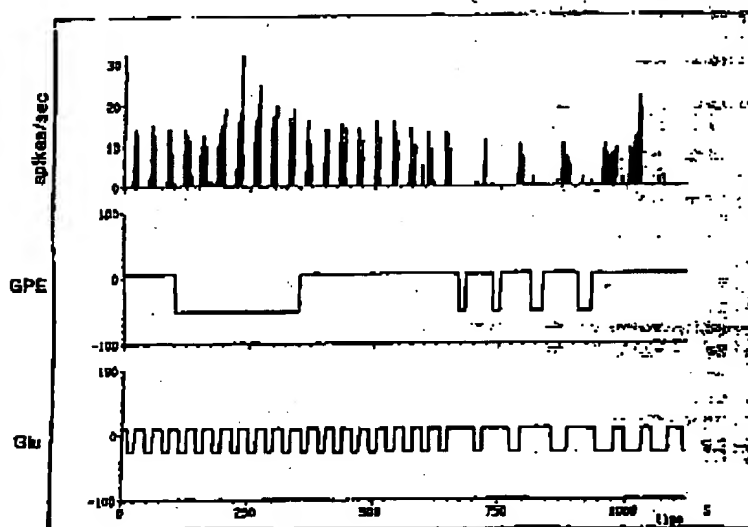
SARA *et al.*: TRUNCATED IGF-1

FIGURE 5. Cortical neurone electrophysiological activity following microiontophoretic application of GPE. The effect of GPE on spontaneous as well as glutamate-driven single cell activity determined as spikes/second is shown.

the glutamate-driven neurone. Similarly, in the spinal cord, GPE has no direct influence on motor neurone activity when applied intrathecally; however, GPE potentiates the facilitated spinal cord reflex in response to other stimuli.

CONCLUSION

Thus there are at least two protein products from expression of the IGF-1 gene in the CNS. These proteins result from posttranslational modification of the IGF-precursor protein. Truncated IGF-1 (-3N:IGF-1) acts as a potent neurotrophic factor and this action is mediated via the IGF-1 receptor. The tripeptide GPE appears to have a quite different CNS function, namely, the modulation of neurotransmitter release. This potentiating action is mediated at least in part via interaction with the NMDA receptor. This is the first example of a product from a growth factor gene being involved in neurotransmission in the CNS. A similarly novel role for the neurotransmitter acetylcholine has been suggested in the regulation of brain growth.

REFERENCES

1. SARA, V. R. & L. LAZARUS. 1974. The prenatal action of growth hormone on brain development. *Nature Lond.* 250: 257-258.
2. SARA, V. R., L. LAZARUS, M. C. STUART & T. KING. 1974. Fetal brain growth: selective action by growth hormone. *Science* 186: 446-447.

3. SARA, V. R., T. L. KING, M. C. STUART & L. LAZARUS. 1976. Hormonal regulation of fetal brain cell proliferation: presence in serum of a trophic responsive to growth hormone stimulation. *Endocrinology* 99: 90-97.
4. SARA, V. R., K. HALL, C. H. BODECK & L. WETTERBERG. 1981. Human embryonic somatomedin. *Proc. Natl. Acad. Sci. USA* 78: 3175-3179.
5. SARA, V. R., C. CARLSSON-SKIRUT, C. ANDERSSON, E. HALL, B. SÖGREN, A. HOLMGREN & H. JÖRNVALL. 1986. Characterization of somatomedins from human fetal brain: identification of a variant of insulin-like growth factor I. *Proc. Natl. Acad. Sci. USA* 83: 4904-4907.
6. CARLSSON-SKIRUT, C., H. JÖRNVALL, A. HOLMGREN, C. ANDERSSON, T. BENGMAN, G. LUNDQVIST, B. SÖGREN & V. R. SARA. 1986. Isolation and characterization of variant IGF-I as well as IGF-2 from adult human brain. *FEBS Lett.* 210: 46-50.
7. SANDBERG NORDQVIST, A.-C., P.-Å. SUNDQVIST, M. LAKE & V. R. SARA. 1992. Characterization of two DNAs encoding insulin-like growth factor I (IGF I) in the human fetus brain. *Mol. Brain Res.* 12: 275-277.
8. REINECKE, M., K. DRAGENBERG, S. FALGOUT & V. R. SARA. 1991. Presence of IGF-I-like peptides in the neuroendocrine system of the Atlantic hagfish, *Myxine glutinosa* (Cyclostomata): evidence derived by chromatography, radioimmunoassay and immunohistochemistry. *Histochemistry* 96: 191-196.
9. REINECKE, M., D. BETZLER, S. FALGOUT, K. DRAGENBERG & V. R. SARA. Occurrence of members of the insulin superfamily in central nervous system and digestive tract of protochordates. *J. Histochem. Cytochem.* (In press.)
10. NAKAMATSU, S., S. J. CHAN, S. FALGOUT & D. F. STEINER. 1991. Evolution of the insulin gene superfamily. Sequence of a preproinsulin-like growth factor cDNA from the Atlantic hagfish. *J. Biol. Chem.* 266(4): 2397-2402.
11. CHAN, S. J., C. CHU-PING & D. F. STEINER. 1990. Evolution of the insulin superfamily: cloning of a hybrid insulin/insulin-like growth factor cDNA from amphioxus. *Proc. Natl. Acad. Sci. USA* 87: 9319-9323.
12. DRAGENBERG, K., M. REINECKE, C. MAAKE, S. GAMMELTOFT, S. FALGOUT & V. R. SARA. Characterization of IGF receptors in lower vertebrates. *Regul. Peptides* 43: 73-81.
13. OGASAWARA, M., K. P. KAREY, H. MARQUARDT & D. A. SIRBASU. 1989. Identification and purification of truncated insulin-like growth factor I from porcine uterus. Evidence for high biological potency. *Biochemistry* 28: 2710-2721.
14. KAREY, K. P., H. MARQUARDT & D. A. SIRBASU. 1989. Human platelet-derived mitogens. I. Identification of insulinlike growth factors I and II by purification and N amino acid sequence analysis. *Blood* 74(3): 1084-1092.
15. KAREY, K. P. & D. A. SIRBASU. 1989. Human platelet-derived mitogens. II. Subcellular localization of insulinlike growth factor I to the α -granule and release in response to thrombin. *Blood* 74(3): 1093-1100.
16. FRANCIS, G. L., L. C. READ, F. J. BALLARD, C. J. BAGLEY, F. M. UPTON, P. M. GRAVESTOCK & J. C. WALLACE. 1986. Purification and partial sequence analysis of insulin-like growth factor-I from bovine colostrum. *Biochem. J.* 233: 207-213.
17. FORBES, B., L. SZABO, R. C. BAXTER, F. J. BALLARD & J. C. WALLACE. 1988. Classification of the insulin-like growth factor binding proteins into three distinct categories according to their binding specificities. *Biochem. Biophys. Res. Commun.* 157: 196-202.
18. BAGLEY, C. J., B. L. MAY, L. SZABO, P. J. MCNAMARA, M. ROSS, G. L. FRANCIS, J. BALLARD & J. C. WALLACE. 1989. A key functional role for the insulin-like growth factor I N-terminal pentapeptide. *Biochem. J.* 259: 665-671.
19. ROSS, M., G. L. FRANCIS, L. SZABO, J. C. WALLACE & F. J. BALLARD. 1989. Insulin-like growth factor (IGF)-binding proteins inhibit the biological activities of IGF-I and IGF-2 but not des-(1-3)-IGF-I. *Biochem. J.* 258: 267-272.
20. CARLSSON-SKIRUT, C., M. LAKE, M. HANMARIN, K. HALL & V. R. SARA. 1989. A comparison of the biological activities of recombinant intact and truncated insulin-like growth factor I (IGF-I). *Biochim. Biophys. Acta* 1011: 192-197.
21. ADRA, A., S. S. JISSAN, R. D'ARCY, P. C. GUNBERG, A. NORDBERG, B. WINBLAD & V. R. SARA. 1989. Insulin-like growth factor I (IGF-I) receptors in the human brain: quantitative autoradiographic localisation. *Brain Res.* 503: 299-301.

22. BONDY, C., H. WERNER, C. T. ROBERTS, JR. & D. LEROTH. 1992. Cellular pattern of type-I insulin-like growth factor receptor gene expression during maturation of the rat brain: comparison with insulin-like growth factors I and II. *Neuroscience* 46: 905-923.
23. SAKA, V. R. & C. CARLSSON-SKIRUT. 1990. Insulin-like growth factors in the central nervous system: biosynthesis and biological role. In *Growth Factors from Genes to Clinical Application*, V. R. Saka, K. Hall & H. Low, Eds., 179-191. Raven Press, New York, N.Y.
24. MCMORRIS, F. A., T. M. SMITH, S. DESALVO & R. W. FURLANETTO. 1986. Insulin-like growth factor I/somatomedin C: a potent inducer of oligodendrocyte development. *Proc. Natl. Acad. Sci. USA* 83: 822-826.
25. BONDY, C. A. 1991. Transient IGF-I gene expression during the maturation of functionally related central projection neurons. *Neuroscience* 41(11): 3442-3455.
26. PAULMAN, S., G. MEYERSON, E. LINDOREN, M. SCHALLING & I. JOHANSSON. 1991. Insulin-like growth factor I shifts from promoting cell division to potentiating maturation during neuronal differentiation. *Proc. Natl. Acad. Sci. USA* 88: 9994-9998.
27. KOMOLY, S., L. D. HUDSON, H. D. WEBSTER & C. A. BONDY. 1992. Insulin-like growth factor I gene expression is induced in astrocytes during experimental demyelination. *Proc. Natl. Acad. Sci. USA* 89: 1894-1898.
28. GIACOBINI, M.-L., L. OLSON & V. R. SARA. 1990. A truncated form of IGF-1 found in human exerts trophic effects on fetal brain tissue grafts. *Exp. Neurol.* 108: 31-37.
29. BACH, M. A., Z. SHEN-ORR, W. L. LOWE, JR., C. T. ROBERTS, JR. & D. LEROTH. 1991. Insulin-like growth factor I mRNA levels are developmentally regulated in specific regions of the rat brain. *Mol. Brain Res.* 18: 43-48.
30. AYER-LELIEVRE, C., P. A. STANBOM & V. R. SARA. 1991. Expression of IGF-1 and IGF-2 mRNA in the brain and cranio-facial region of the rat fetus. *Development* 111: 105-115.
31. DRAKENBERG, K., C.-G. ÖSTENSON & V. R. SARA. 1990. Circulating forms and biological activity of intact and truncated insulin-like growth factor I (IGF-I) in adult and neonatal rat. *Acta Endocrinol.* 123: 43-50.
32. BALLARD, F. J., S. E. KNOWLES, P. C. OWENS, P. C. OWENS, M. A. MOHLEK & B. L. FERRAROLO. 1991. Plasma clearance and tissue distribution of labelled insulin-like growth factor-I (IGF), IGF-II and des(1-3)IGF-I in rats. *J. Endocrinol.* 128: 197-204.
33. GULLISPIE, C., L. C. READ, C. J. BAILEY & F. J. BALLARD. 1990. Enhanced potency of truncated insulin-like growth factor-I (des(1-3)IGF-I) relative to IGF-I in *hiv* mice. *J. Endocrinol.* 127: 401-405.
34. TOMAS, P. M., S. E. KNOWLES, P. C. OWENS, L. C. READ, C. S. CHANDLER, S. E. GARODSKY & F. J. BALLARD. 1990. Effects of full-length and truncated insulin-like growth factor-I on nitrogen balance and muscle protein metabolism in nitrogen-restricted rats. *J. Endocrinol.* 128: 97-105.
35. SARA, V. R., C. CARLSSON-SKIRUT, T. DEROMAN, H. JÖRNVALL, P. J. ROBERTS, M. CRANFORD, L. NILSSON-HÅKANSSON, I. CIVALEO & A. NORDBERG. 1989. Identification of the truncated aminoterminal tripeptide of IGF-1, Gly-Pro-Glu (GPE), as a novel neuroactive peptide. *Biochem. Biophys. Res. Commun.* 163: 766-771.
36. RANSOM, R. W. & N. L. DISCHENES. 1989. Glycine modulation of NMDA-evoked release of ³H-acetylcholine and ³H-dopamine from rat striatal slices. *Neurosci. Lett.* 96: 323-328.
37. NILSSON-HÅKANSSON, L., I. CIVALEO, X. ZHANG, C. CARLSSON-SKIRUT, V. R. SARA & A. NORDBERG. Neuroactive products of IGF-1 gene expression: action on ³H choline efflux in rat cortex. *Eur. J. Pharmacol.* (Submitted.)
38. NILSSON, L., V. R. SARA & A. NORDBERG. 1988. Insulin-like growth factor I stimulates the release of acetylcholine from rat cortical slices. *Neurosci. Lett.* 88: 221-226.
39. DAHMER, M. K., P. M. HART & R. L. PERLMAN. 1990. Studies on the effect of insulin-like growth factor-I on catecholamine secretion from chromaffin cells. *J. Neurochem.* 54: 931-936.
40. ASHKTANZI, A., J. RAMACHANDRAN & D. J. CAPON. 1989. Acetylcholine analogue promotes DNA synthesis in brain-derived cells via specific muscarinic receptor subtypes. *Nature* 340: 146-150.